REMARKS

This paper is filed in response to the Office Action mailed on October 20, 2004. Currently, Claims 1-20 are pending. Of these, Claims 17-20 are withdrawn from consideration. Claims 3-5 have been canceled without prejudice to filing a continuing application to the subject matter of the canceled claims. Accordingly, reconsideration and allowance of Claims 1, 2, and 6-16, is respectfully requested.

The Objection to the Specification

The specification is objected to because of a spelling error. The specification has been amended to correct the error. Accordingly, withdrawal of the objection is respectfully requested.

The Rejection of Claim 11 Under 35 U.S.C. § 112, First Paragraph

Claim 11 is rejected under 35 U.S.C. § 112, first paragraph.

Claim 11 has been amended to recite "heating the extract to between about 45 to 70°C to increase the specific activity of PDE-1 in the extract." Accordingly, withdrawal of the rejection of Claim 6 is respectfully requested.

The Rejection of Claims 1, 4, 5, and 7-10 Under 35 U.S.C. § 102(b)

Claims 1, 4, 5, and 7-10 are rejected under 35 U.S.C. § 102(b) as being anticipated by Holle et al. (U.S. Patent No. 3,516,907).

Claim 1 has been amended. Claims 4 and 5 have been canceled. Claims 7-10 depend from Claim 1.

As now amended, Claim 1 recites "wherein the concentration of the divalent cation is about 10 to 50 mM."

For a reference to be anticipatory, the reference must exactly describe the claimed invention.

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CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

Prior to addressing the Examiner's rejection, applicant provides a brief description of the invention.

Phosphodiesterase 1 (PDE-1) is an enzyme that catalyses the hydrolysis of a phosphodiester bond at the 3' hydroxy end of a ribopolynucleotide to yield a 5' ribonucleotide. Purified forms of this enzyme are valuable because they can be used to produce high value flavor enhancers from low value products. Given this, there has, for some time, been a need to obtain purified forms of PDE-1.

At the time of the present invention, it was basically not known how to purify PDE-1 from a cellular extract. At best, all that could be achieved was the obtainment of a crude extract including a *class* of enzymes, such as diesterases, monoesterases, ribonucleases, deoxyribonucleases, nucleotidases, and nucleosidases. U.S. Patent No. 3,516,907 (Holle et al.) confirms this point:

> While it is possible to use the crude enzyme extract, it is generally preferred to enrich the enzyme concentration by the simple purification steps . . . Any 5'-nucleotidase, an enzyme which would split phosphoric acid from the 5'-mononucleotides, which is still present is then removed by subsequent chromatographic separation. (Col. 2, line 62 to Col. 3, line 2.)

According to the present application, it has been found how to selectively purify PDE-1 from a cellular extract. In particular, it has been found that by applying at least one divalent cation having a concentration range as defined in amended Claims 1 and 11 and heating, it is possible to increase the specific activity of PDE-1, and hence, to purify PDE-1 in an extract. The present application expressly teaches the importance of the divalent cation in the context of protecting PDE-1 from denaturation at temperatures at which other proteins in an extract, including monoesterases, and other enzymes for forming a mononucleotide noted above, are denatured (page 3, lines 10 to 12). This finding is of importance because it permits heat

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1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

treatment, a step that is relatively simple to operate on a commercial scale to be implemented with minimal loss of PDE-1 (page 3, lines 6 to 9).

The lower concentration range over which at least some improvement in protection from denaturation is observed has been found to be about 10 mM of at least one divalent cation. These low concentration ranges are advantageous because it has also been found in the present application that high concentrations of cations inhibit adsorbance of PDE-1 to an anion exchanger. Thus low concentration ranges facilitate further purification steps as these are required (page 3, lines 21 to 24).

To summarize the above, the present application teaches the combination of particular concentrations of divalent cations and temperature for selective purification of PDE-1 from an extract. It also teaches the importance of cations in this process.

Turning to Holle et al., this document does not disclose, teach, or suggest a concentration range of the at least one divalent cation as in amended Claim 1, or the concentration ranges of magnesium and calcium in amended Claim 11. On this point, applicant respectfully traverses the Examiner's comment "*5 mM is defined by the examiner to about 10 mM.*" Applicant respectfully submits that 10 mM is 2 fold more than 5 mM, and this difference is significant in terms of the extent to which PDE-1 is protected from heat denaturation. Example 10, page 11, 17 to 20 of the present application supports this submission:

> We found that after heating, the activity dropped significantly in samples without magnesium and calcium and in samples with 5 mM $MgCl_2$ and 5 mM $CaCl_2$ by over 40% . . . .

Applicant respectfully notes that Holle et al. do not teach or suggest the finding of the present application that the divalent cation has a role in protecting PDE-1 from heat denaturation. Indeed, Holle et al. teach that the divalent cation is not essential to the invention, but merely only optional. See, for example, Col. 4, lines 3-6:

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1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

> If it is desired to work without the addition of a heavy metal salt, then it is preferred to inactivate the 5'-nucleotidase by adjusting the pH of the extract to between 4 and 7.

Examples 1 to 22 of Holle et al., which discuss preparation of a crude extract without salt, support this submission.

Given the above, applicant respectfully submits that 5 mM is not about 10 mM. Therefore, because Holle et al. do not teach at least "wherein the concentration of the divalent cation is about 10 to 50 mM," the reference is not anticipatory. Applicant submits that doubling the upper limit of 5 mM is not about 10, as claimed. There being no other discussion in Holle et al. as to concentration ranges of a divalent cation that are higher than 5 mM, applicant respectfully submits that Claims 1, 4, 5, and 7-10 are novel over Holle et al. and that the rejection should be withdrawn.

The Rejection of Claims 1-5 and 7-16 Under 35 U.S.C. § 103(a)

Claims 1-5 and 7-16 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Holle et al. in view of Harvey et al. (*Biochemsitry*, 1967) and De-Eknamkul et al. (U.S. Patent No. 5,879,916), and in light of Hsin et al. (*Blood* 1998).

Claim 1 and Claim 11 have been amended. Claims 3-5 have been canceled. Claims 2, and 7-10 depend directly or indirectly from Claim 1, and Claims 12-16 depend directly or indirectly from Claim 11.

As now amended, Claim 1 recites, "wherein the concentration of the divalent cation is about 10 to 50 mM." Claim 11 recites "releasing PDE-1 from the cell into a solution including about 10 to 50mM calcium and about 10 to 50mM magnesium to form an extract; and heating the extract to between about 45 to 70°C to increase the specific activity of PDE-1 in the extract."

A *prima facie* case of obviousness requires a suggestion or motivation either in the references or in the knowledge generally available to modify a reference or to combine

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1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

references, a reasonable expectation of success, and all the claim elements must be found in the references.

The Examiner states that:

> though Holle et al. describes using between 2 mM and 5 mM of heavy metal salts, it would have been obvious to one of ordinary kill in the art to experiment with slightly higher concentrations of metal salts up to and including a concentration of 10 mM (Claim 5). One would be motivated to manipulating the concentration within a reasonable range, such as +/- 5 mM, because the references clearly indicates that the various proportions and amounts of the ingredients used in the claimed composition are result effective variables, they would be routinely optimized by one of ordinary skill in the art in practicing the invention disclosed by those references.

Applicant respectfully disagrees with the Examiner.

The Examiner never states where the reference or references provide the supposed indication that the divalent cation is a "result effective variable." A general allegation of what the reference teaches is not helpful in furthering the prosecution of the present application. As discussed above, Holle et al. teach that a "heavy metal salt" is merely only optional. (See Col. 4, lines 3-6.) Furthermore, even if one were to include a heavy metal salt, "the concentration of the heavy metal salts in the extracts are preferably limited to between 10^{-2} and 10^{-5} molar concentrations." (See Col. 3, lines 46-48.) The words "preferably limited" do not remotely suggest or motivate one to experiment outside of the stated ranges of Holle et al. The Examiner must weigh all the teachings of the references. If the references substantially teach away from performing the claimed invention, the claims cannot be considered obvious therefrom. Accordingly, for the reasons above, the heavy metal salt cannot be considered a "result effective variable," and furthermore Holle et al. clearly teach away from raising the concentration of the heavy metal salt above 5 mM.

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CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

Additionally, Holle et al. do not teach or suggest that the heating step is to be used for denaturation of enzymes. Indeed, Holle et al. do not teach the purpose of the heating step at all.

Further, as noted above, Holle et al. do not teach or suggest that the heavy metal salts have a role in protecting PDE-1 from heat denaturation.

Rather:

- Holle et al. discuss that steps other than the heating step are to be used, for example, further chromatographic separation (Col. 2, line 62 to Col. 3, line 2), or pH adjustment (Col. 3, lines 60-64, lines 70-74, and Col. 4, lines 3-6) for enriching the concentration of a relevant enzyme.
- Holle et al. at Col. 4, lines 3-6, and Examples 1-22 discuss preparing a crude extract without use of heavy metal salts, suggesting a failure of Holle et al. to recognize the importance of the salts discussed therein for protecting PDE-1 during heating.

Given the above, it must follow that it would not have been obvious to one of ordinary skill in the art in reading Holle et al. to experiment with higher concentrations of salts as claimed. Holle et al. simply provide no teaching or suggestion on the purpose of heating and heavy metal salts, and accordingly gives no reason for the skilled person to use a higher concentration of salt as per Claims 1 and 11. Accordingly, Holle et al. provide no motivation to one skilled in the art to manipulate the concentration of the heavy metal salts.

Further, as none of the other cited references supplement the above deficiency in Holle et al., it is respectfully submitted that when combined with Holle et al., it would not have been obvious for one skilled in the art to experiment with higher concentrations of salts. Accordingly, the withdrawal of the rejection of Claims 1, 2, and 7-16 is respectfully requested.

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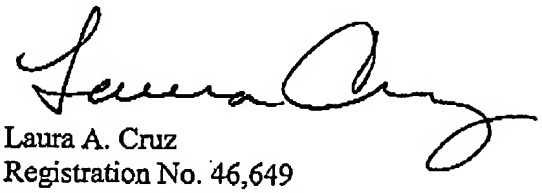
CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

CONCLUSION

In view of the foregoing amendments and remarks, applicant respectfully submits that Claims 1, 2, and 6-16 are in condition for allowance. If there are any questions or comments, the Examiner is encouraged to contact the applicant's attorney at the number provided below.

Respectfully submitted,

CHRISTENSEN O'CONNOR
JOHNSON KINDNESS PLLC



Laura A. Cruz
Registration No. 46,649
Direct Dial No. 206.695.1725

I hereby certify that this correspondence is being transmitted via facsimile to the U.S. Patent and Trademark Office, Group Art Unit 1651, Examiner Allison M. Ford, at facsimile number 703.872.9306 on January 20, 2005.

Date: January 20, 2005 Victoria Sellers

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CHRISTENSEN O'CONNOR JOHNSON KINDNESS PLLC
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100